REMARKS

Reconsideration of the above-identified application in view of the amendment proposed above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claim 1 has been amended in this paper. Therefore, claims 1-12, 14-18, 20-23, 25-26, 30-34, 36 and 41 are pending and are under active consideration.

Claims 1-8, 10-11, 14-15, 17-18, 20, 22, 23, 25-26, 30-34 and 41 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to repeating the method of claim 1 which is drawn to a method of determining differential gene expression, and analyzing methylation states of the gene, wherein the methylation state is determined by use of a bisulfite reaction.
>
> Kikyo et al. shows in the abstract and throughout a method of analysis of mouse embryo tissue for genes that are differentially expressed between normal embryos and abnormal embryos with chromosomal translocations. A differentially expressed neuronatin (Nnat) gene was shown to be imprinted by methylation analysis. Kikyo et al. shows on pages 68-69 differential display analysis of mRNA from the embryos, in which eighty primer pairs were used, and approximately 80-100 bands per primer pair were observed. Ten differentially expressed bands corresponding to differentially expressed genes were observed. Two genes were identified as H19 and Nnat (see figures 1A and 1B). Kikyo et al. further noted on page 69 prior art that used subtraction hybridization to identify Nnat as a differentially expressed gene, and verified Nnat differential expression by a reverse transcriptase-polymerase chain reaction method (see figure 1C). Kikyo et al. subsequently analyzed the Nnat

gene for methylation by digestion with a panel of restriction endonucleases Hind III, BssH II, Eag I, and Sac II (see figure 6).

The New England Biolab website establishes that BssH II, Eag I and Sac II enzymes are inhibited by methylation at CpG sites.

Siegfried et al. establishes on page R305 that CpG methylation is a term of art meaning that a cytosine is methylated.

Kikyo does not show repetition of steps or use of a bisulfite reaction to determine methylation states of cytosine.

Frommer et al. shows in the abstract and throughout a method to determine the positions of methylated cytosine residues in DNA by use of sodium bisulfite to convert cytosine to uracil in a chemical reaction (which does not react with methylated cytosine). Frommer et al. shows in page 1828 and figure 1 that their method comprises polymerase chain reactions subsequent to the sodium bisulfite treatment that produces polynucleotides suitable for sequencing reaction analysis. The sequence analysis of the amplified products reveals the presence of positions that originally contained methylated cytosine (see figures 2 and 3). Frommer et al. lists advantages of their method on page 1830, including the positive display of methylated cytosine residues, and the capacity to analyze individual strands of a DNA sample.

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to repeat the steps of Kikyo et al. for the purpose of analysis of additional tissue and genes for determination of correlations between expression and methylation, as shown by Kikyo et al. It would have been further obvious to modify the method of Kikyo et al. by use [of] the sodium bisulfite reaction method of methylated cytosine detection of Frommer et al. because Frommer et al. shows that their method also detects methylated cytosine, and has further advantages of positive display of methylated cytosines residues and the ability to analyze individual strands of a DNA sample.

Applicants respectfully traverse the subject rejection. Claim 1, from which claims 2-8, 10-11, 14-15, 17-18, 20, 22-23, 25-26, 30-34 and 41, has been amended herein and now recites "[a] method for the development of gene panels for diagnostic and therapeutic purposes, comprising the steps of:

a) isolating at least one biological sample from each of at least two groups of biological material containing mRNA and/or proteins, wherein said groups are either healthy and cancer or cancer;

b) analysing the expression level of at least one gene in at least one of the biological samples;

c) selecting the gene(s) exhibiting a different expression level between said at least two groups of biological material, whereby a first knowledge base is generated;

d) analysing the level of cytosine methylation of at least one gene of said first knowledge base in at least one of the biological samples of step a) by means comprising treatment with bisulphite, hydrogen sulphite or disulphite;

e) selecting gene(s) exhibiting a different level of cytosine methylation between said at least two groups of biological material, whereby a second knowledge base is generated; and

f) adding selected genes from the second knowledge base to a gene panel."

Claim 1 is not unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. for at least the reason that none of these references, taken individually or in combination, teaches or suggests, among other things, the claimed step of isolating at least one biological sample from each of at least two groups of biological material containing mRNA and/or proteins, wherein said groups are either healthy and cancer or cancer. Instead, Kikyo

et al. discloses the comparison of healthy mouse embryos to those displaying developmental, neurological and behavioural abnormalities. Kikyo et al. does not disclose the comparison of healthy and cancer groups. The other references do not cure this deficiency. For example, Frommer et al. discloses the comparison of healthy tissues only. No document discloses the analysis of cancer samples.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1, 6, 9, 16, 21 and 36 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above and further in view of Danssaert et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to the method of claim 1 with the further limitation that the methylation analysis comprises use of a robot or a computer device.
>
> Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above does not show a methylation analysis that comprises use of a robot or a computer device.
>
> Danssaert et al. shows in column 1, lines 22-25 that polymerase chain reactions are best performed on automated devices that allow for consistent thermal cycling. Danssaert et al. shows computer controlled thermal cyclers that comprise robotic arms in column 1, line 33, column 4, and lines 39-50, column 5, lines 31-48.
>
> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the method of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by use of a computer

> controlled thermal cycler, optionally with robotic arms, for conducting the polymerase chain reactions because Danssaert et al. shows that automated thermal cyclers have the advantage of providing consistent thermal cycling, and further because it is obvious to automate a manual activity (see MPEP 2144.04).

Applicants respectfully traverse the subject rejection. Claim 1 is patentable over Kikyo et al., New England Biolabs, Siegfried et al. and Frommer et al. for at least the reasons above. Danssaert et al. fails to cure all of the deficiencies of these references. Therefore, claim 1 is patentable over the applied combination of references. Claims 6, 9, 16, 21 and 36 depend from claim 1 and are patentable over the applied combination of references based at least on their respective dependencies from claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 1 and 12 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above, and further in view of Anderson et al." In support of the rejection, the Patent Office states the following:

> The claims are drawn to the method of claim 1 with the further limitation that both mRNA and protein levels are measured.
>
> Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above does not show measurement of protein levels.
>
> Anderson et al. shows comparison of human liver gene expression by measurement of mRNA levels and corresponding protein levels (as measured by two-dimensional protein electrophoresis). Anderson et al. shows moderate levels of correlation between mRNA levels and protein levels in figures 1 and

> 2. Anderson et al. conclude on page 537 that determination of protein levels allows for a better understanding of multi-level gene expression control in complex organisms such as man.
>
> It would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify of Kikyo et al. as evidenced by New England Biolabs and Siegfried et al. in view of Frommer et al. as applied to claims 1-8, 10, 11, 14, 15, 17, 18, 20, 22, 23, 25, 26, 30-34, and 41 above by additional use of the protein analysis method of Anderson et al. because Anderson et al. shows that determination of correlations between mRNA and protein levels allows for better understanding of gene expression controls.

Applicants respectfully traverse the subject rejection. Claim 1 is patentable over Kikyo et al., New England Biolabs, Siegfried et al. and Frommer et al. for at least the reasons above. Anderson et al. fails to cure all of the deficiencies of these references. Therefore, claim 1 is patentable over the applied combination of references. Claim 12 depends from claim 1 and is patentable over the applied combination of references based at least on its dependency from claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on November 24, 2006.

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